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ORIGINAL REPORT

Survival Benefit With Proapoptotic Molecular and Pathologic Responses From Dual Targeting of Mammalian Target of Rapamycin and Epidermal Growth Factor Receptor in a Preclinical Model of Pancreatic Neuroendocrine Carcinogenesis

Christopher W. Chiu, Hiroaki Nozawa, and Douglas Hanahan

See accompanying editorial on page 4407

From the University of California, San Francisco, CA.

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Corresponding author: Douglas Hanahan, PhD, Swiss Institute for Experimental Cancer Research, Swiss Federal Institute of Technology Lausanne, Life Sciences Building (SV), Rm SV-2816, Lausanne, CH-1015, Switzerland; e-mail: dh@epfl.ch.

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ABSTRACT

Purpose
Pancreatic neuroendocrine tumors (PNETs), although rare, often metastasize, such that surgery, the only potentially curative therapy, is not possible. There is no effective systemic therapy for patients with advanced PNETs. Therefore, new strategies are needed. Toward that end, we investigated the potential benefit of dual therapeutic targeting of the epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (mTOR) kinases, using a preclinical mouse model of PNET.

Materials and Methods
Rapamycin and erlotinib, inhibitors of mTOR and EGFR, respectively, were used to treat RIP-Tag2 transgenic mice bearing advanced multifocal PNET. Tumor growth and survival were monitored, and tumors were surveyed for potential biomarkers of response to the therapeutics.

Results
Rapamycin monotherapy was notably efficacious, prolonging survival concomitant with tumor stasis (stable disease). However, the tumors developed resistance, as evidenced by eventual relapse to progressive tumor growth. Erlotinib monotherapy slowed tumor growth and elicited a marginal survival benefit. In combination, there was an unprecedented survival benefit in the face of this aggressive multifocal cancer and, in contrast to either monotherapy, the development of adaptive resistance was not apparent. Additionally, the antiapoptotic protein survivin was implicated as a biomarker of sensitivity and beneficial responses to the dual targeted therapy.

Conclusion
Preclinical trials in a mouse model of endogenous PNET suggest that combined targeting of the mTOR and EGFR signaling pathways could have potential clinical benefit in treating PNET. These results have encouraged development of an ongoing phase II clinical trial aimed to evaluate the efficacy of this treatment regimen in human neuroendocrine tumors.

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INTRODUCTION

Pancreatic neuroendocrine tumors (PNETs/islet cell tumors) have a low (1% to 2%) incidence yet a 10% prevalence among pancreatic cancers and are often diagnosed at an advanced stage, with limited treatment options after failure of chemotherapy.[1,2] Therefore, there is need for new therapies. In this study, we investigated in a preclinical model of PNET two distinct but interconnected cancer signaling pathways: the epidermal growth factor receptor (EGFR/ErbB1; human epidermal growth factor receptor 1 in humans) and the mammalian target of rapamycin (mTOR).

mTOR is a ubiquitous, highly conserved serine/threonine kinase that regulates a number of cellular functions, including protein synthesis and cell proliferation, and is activated in many cancers.[3-5] Rapamycin is a potent and specific inhibitor of mTOR and has been shown to inhibit tumor growth, angiogenesis, and metastasis, as well as induce apoptosis in cancer cell lines and in mouse models of cancer.[6-8] Two rapamycin analogs ("rapalogs"), everolimus and temsirolimus,

have been approved for treatment of advanced renal cell carcinoma after failure of chemotherapy.[9] In PNET, everolimus has been shown to have efficacy against metastatic PNET after failure of cytotoxic chemotherapy in a phase II trial and is being evaluated in a phase III trial as a first-line option for treating PNET.[1,10,11] A mechanism of adaptive resistance to mTOR inhibitors has been described, involving loss of mTOR-dependent feedback inhibition of an upstream signaling molecule, the Akt kinase,[12-14] whose heightened activity can circumvent some of the effects of mTOR inhibition.

EGFR signaling affects a number of capabilities in tumors, including proliferation, survival, angiogenesis, and invasion. Overexpression and/or increased activity of EGFR is common and is correlated with decreased survival in multiple forms of human cancer; among its downstream signal transducers is the aforementioned Akt kinase. EGFR inhibitors, including erlotinib, have been approved for pancreatic ductal cancer and non–small-cell lung cancer.[15] An EGFR inhibitor, gefitinib, has been shown to have efficacy against progressive metastatic PNET in a phase II trial.[16] The efficacy of EGFR inhibitors is typically transitory due to the development of various forms of resistance.[17-19]

Several considerations led us to assess inhibiting EGFR and mTOR, alone and in combination, in preclinical trials for PNET. First, our pilot studies with rapamycin, and a parallel study with erlotinib,[20] showed that each drug had efficacy in the PNET model. Second, we hypothesized that adaptive resistance to rapamycin might also involve upregulation of Akt, and if so, then the resistance might be abrogated by erlotinib, because EGFR activates Akt in this model.[20]

The third rationale for this preclinical investigation was teleologic: the growing armamentarium of targeted therapies, rational combinations, and sophisticated regimens raises a daunting logistical challenge in terms of performing instructive clinical trials, with the attendant necessity to prioritize those with the best prospect for success. Arguably, preclinical trials in representative mouse models of the human cancers may present one avenue to evaluate mechanism-based drugs and trial designs. Indeed there is reason to be cautiously optimistic that mechanism-based preclinical trials in genetically engineered mouse models of cancer can both encourage clinical trials and be predictive of benefit. Consider for example the publication in this journal of a novel "chemo-switch" regimen, developed in the prototypical RIP1-Tag2 transgenic mouse model of PNET,[21] involving high-dose chemotherapy (mimicking standard-of-care) followed by a regimen of metronomic (antiangiogenic) chemotherapy combined with a specific angiogenesis inhibitor; this regimen elicited partial responses and demonstrable survival benefit.[22] Recent results of a conceptually analogous phase II clinical trial in human renal cancer reported clinical benefit,[23,24] encouraging both further clinical investigation of the chemo-switch trial design in renal and other cancers and, in the broader sense, the use of genetically engineered mouse models of human cancer types to evaluate the effects of mechanism-based targeting strategies and thereby help prioritize and refine clinical trial designs. The present study continues that approach.

We report below significant benefit to dual targeting of EGFR and mTOR, which in combination substantively reduced tumor burden and extended life expectancy in mice with advanced PNET. The preclinical trials described herein have motivated an ongoing phase II clinical trial to test the safety and efficacy of this combined therapy in treating patients with neuroendocrine tumors.

MATERIALS AND METHODS

Animals

The University of California, San Francisco committee for animal care approved all animal studies described. From 12 weeks of age, RIP1-Tag2 mice received 50% sugar food (Harlan, Madison, WI) to relieve hypoglycemia induced by the insulin-secreting tumors.

Experimental Trials

Rapamycin (sirolimus; Rapamune; Wyeth, Madison, NJ) and erlotinib (Tarceva; Genentech, South San Francisco, CA) were purchased (University of California, San Francisco pharmacy). Erlotinib was crushed and resuspended daily in vehicle (0.4% Tween-20, 150 mmol/L of NaCl and 0.5% carboxymethyl cellulose). The animals were dosed daily by oral gavage with rapamycin in the morning and erlotinib in the afternoon. The animals were weighed biweekly and monitored daily for signs of distress and cachexia and euthanized if the animals became moribund.

The pancreases of the mice were dissected, and macroscopic tumors (> 0.5 mm^3) were counted and measured. Tumor volume (v) was calculated by using the formula for a spheroid: $v = 0.52 \times (\text{width})^2 \times (\text{length})$.

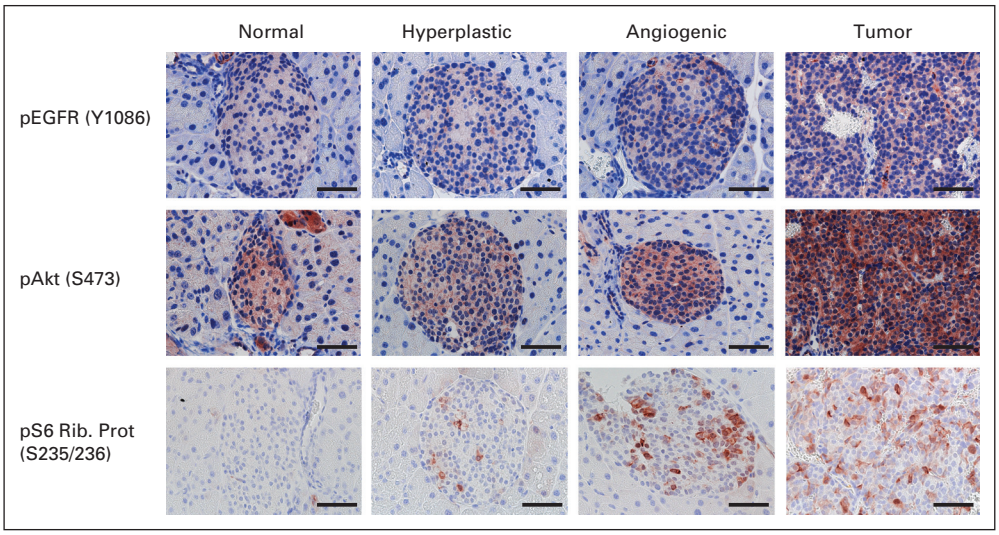


Fig 1. Progressive activation of the Akt/mammalian target of rapamycin (mTOR) and epidermal growth factor receptor (EGFR) signaling pathways during pancreatic neuroendocrine tumor tumorigenesis in RIP1-Tag2 mice. Pancreases were dissected from 5-week-old wild-type and from 5-, 7.5-, and 12-week-old RIP1-Tag2 animals representing the hyperplastic, angiogenic, and tumor stages, respectively. Immunostaining was performed with antibodies that are indicative of activation of EGFR, mTOR, and Akt: phosphorylated EGFR (pEGFR-Y1086), phosphorylated S6 ribosomal protein (Rib. Prot; pS6–S235/236), and phosphorylated Akt kinase (pAkt–S473), respectively. Representative lesions are shown. Scale bars, 50 μm.

The sum of all the tumor volumes from each mouse was calculated as the tumor burden.

Tissue Preparation and Immunostaining

Tissues were paraffin-embedded[25] and immunohistochemistry and terminal deoxynucleotidyl transferase dUTP nick end labeling staining was as previously described.[26] Primary antibodies included pan-Akt, pAkt (ser473), pEGFR (tyr1068), pS6 ribosomal protein (Ser235/236) and survivin (Cell Signaling technology, Beverly, MA), anti-CD31 and anti-Meca-32 (BD Biosciences, San Jose, CA), anti-Desmin (Abcam, Cambridge, MA), and anti-Ki67 (Novus, Littleton, CO). The sections were developed using DAB (Sigma-Aldrich, St Louis, MO) or NovaRed (Vector labs, Burlingame, CA) and counterstained with methyl green or hematoxylin.

Viability, Apoptosis, and Cell Cycle Analysis

βTC3 cells were transfected with *Birc5* or control siRNAs (Dharmacon, Lafayette, CO, L-043690-00-0005) using Lipofectamine 2000, and analyzed with the MTT cell viability (Invitrogen, Carlsbad, CA) and Annexin V apoptosis (BD Biosciences) assays performed according to the manufacturer's protocols. For cell cycle analysis, the cells were fixed with ice-cold 70% ethanol, washed with phosphate-buffered saline, and incubated with 20 μg/mL of propidium iodide, 200 μg/mL of RNase A in 0.1% Triton X-100/phosphate-buffered saline, and analyzed by fluorescence activated cell sorting.

RESULTS

Upregulation of the mTOR and EGFR Signaling Pathways During PNET Tumorigenesis in Rip1-Tag2 Oncomice

We investigated the mTOR and EGFR signaling pathways in a genetically engineered mouse model of PNET, the well-characterized RIP1-Tag2 model,[21] motivated both by the growing appreciation of the roles that these interconnected signaling pathways play in cancer and by the availability of clinically approved inhibitors.[7,27-30] These mice synchronously progress from incipient neoplasia to invasive carcinomas, enabling us to audit the activation status of the mTOR and EGFR pathways in each of the distinctive stages of PNET tumor development. Additionally, we assessed activation of the Akt kinase, a signal transducer involved in both signaling circuits.

In normal pancreas, there was little detectable activation of the mTOR pathway and modest activation of EGFR and Akt (Fig 1). During RIP1-Tag2 tumor development, we observed progressive and robust activation of mTOR, EGFR, and Akt, suggesting that these signaling pathways might have functionally important roles.

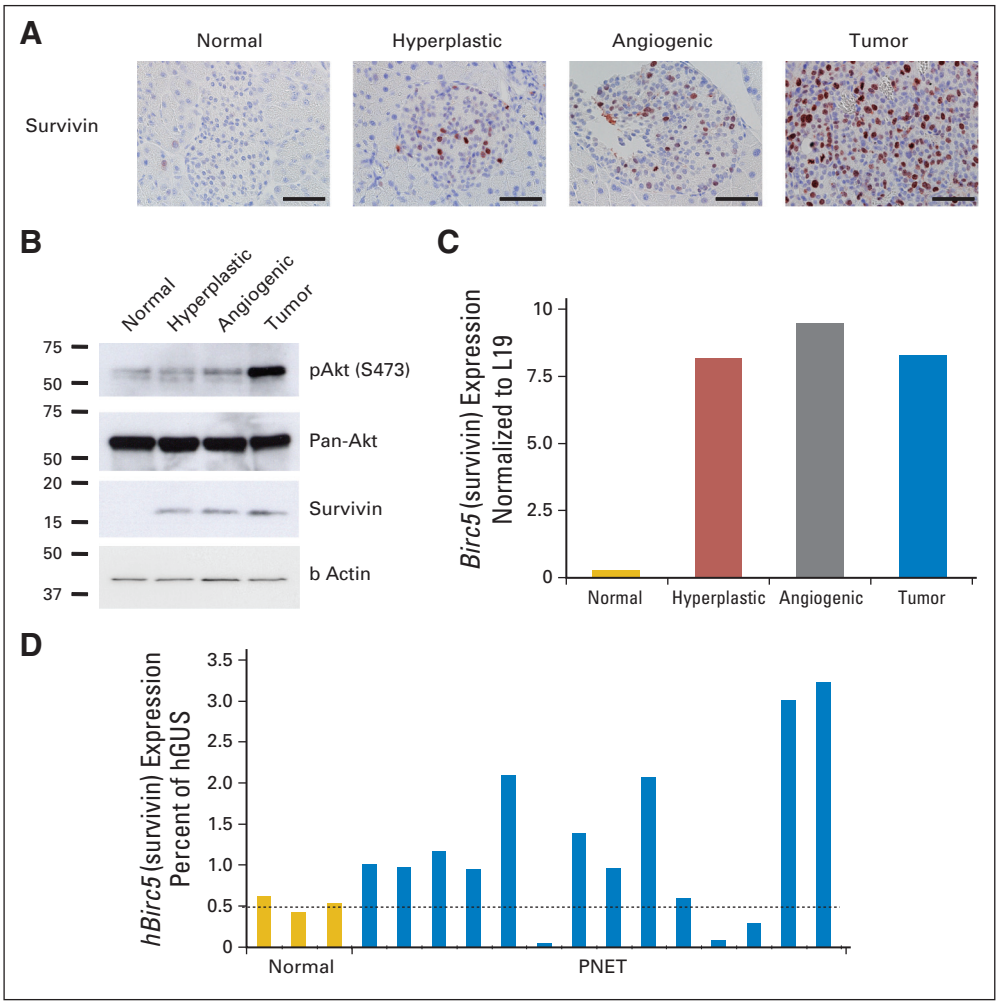


Fig 2. Increased expression of survivin during tumor progression in RIP1-Tag2 mice and in human pancreatic neuroendocrine tumors (PNETs). (A) Immunostaining of representative lesions for expression of *Birc5* (survivin) in the hyperplastic, angiogenic, and tumor stages. Scale bars, 50 μm. (B) Western blot and (C) quantitative polymerase chain reaction (PCR) analysis confirms increased expression of *Birc5* during PNET tumor progression in RIP1-Tag2 transgenic mice. (D) Quantitative PCR for human *Birc5* (*hBirc5*) from three samples of normal islets and 14 human PNETs. The data were normalized to the control gene human β-glucuronidase (hGUS).

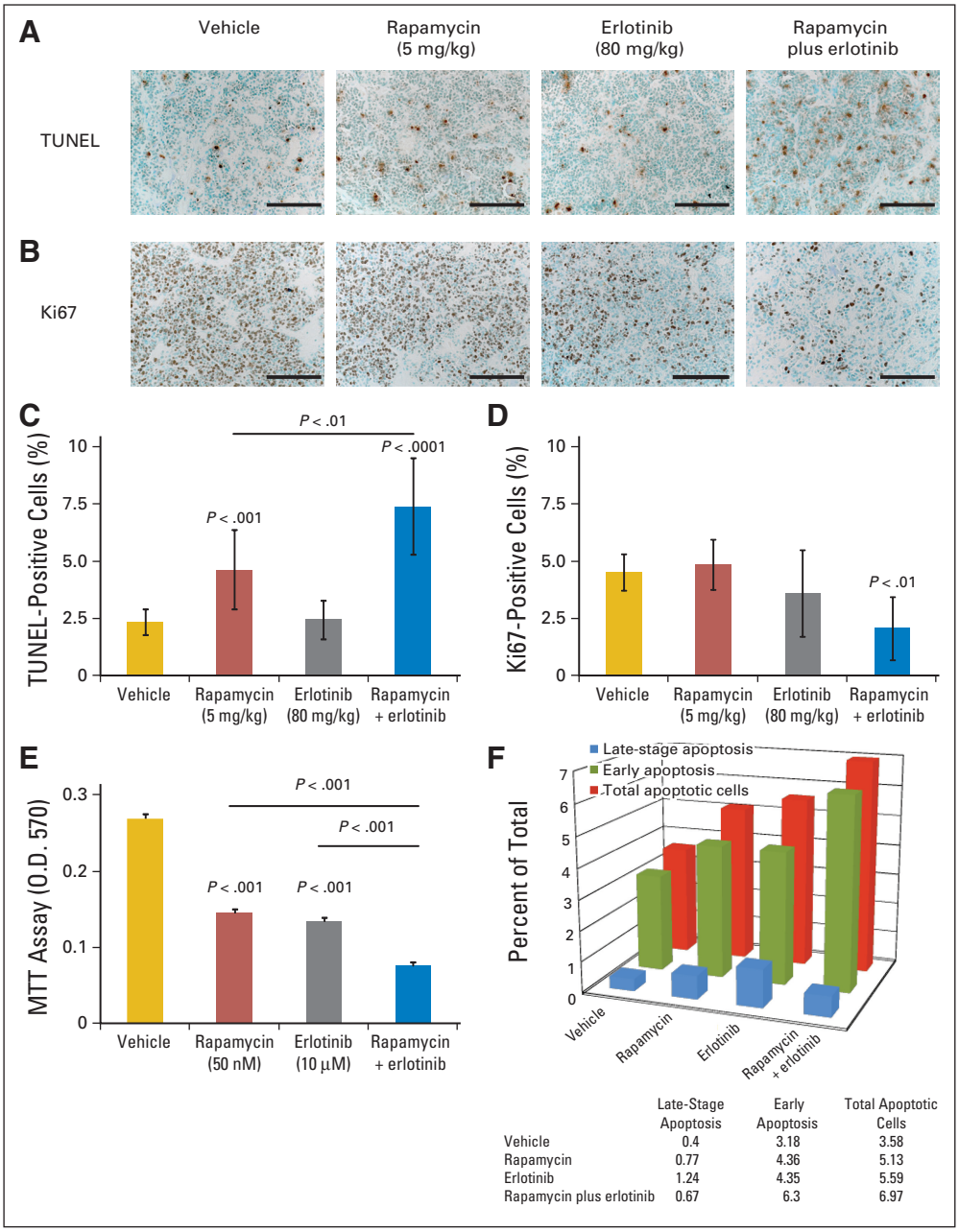
Because both mTOR and EGFR signaling pathways can modulate the expression of apoptotic regulatory factors, we surveyed expression of the inhibitors of apoptosis proteins and Bcl2 families of survival factors and among these, only the expression of the inhibitors of apoptosis proteins member *Birc5* (survivin) was significantly increased during tumor progression (Figs 2A through 2C, and data not shown). Notably, survivin is upregulated in multiple cancer types and has been proposed as a potential therapeutic target due to its restricted expression in adult tissues.[31-34]

As an initial entrée into the possible relevance of these observations to human PNET, quantitative polymerase chain reaction analysis was performed on samples derived from normal human islets and a collection of human PNET.[35] A majority of the human PNET samples tested (10 of 14) had elevated *Birc5* expression (Fig 2D), suggesting a commonality with the mouse PNET. This result is consistent with previous studies of human neuroendocrine tumors, where elevated survivin expression was observed, and proposed as a predictive biomarker of cancer progression and survival.[36-41]

Molecular Efficacy (Phase Zero) Trials Involving Dual Inhibition of the mTOR and EGFR Signaling Pathways

Before initiating long-term efficacy trials, we assessed target modulation and combinatorial effects in tumor-bearing RIP1-Tag2 mice. We selected two clinically approved inhibitors: the EGFR inhibitor erlotinib and the mTOR inhibitor rapamycin. RIP1-Tag2 animals were treated for 3 days with vehicle: rapamycin at 5 mg/kg and erlotinib at 80 mg/kg or in combination.

Histochemical staining for cells undergoing programmed (apoptotic) cell death revealed that the short-term rapamycin treatment



	Late-Stage Apoptosis	Early Apoptosis	Total Apoptotic Cells
Vehicle	0.4	3.18	3.58
Rapamycin	0.77	4.36	5.13
Erlotinib	1.24	4.35	5.59
Rapamycin plus erlotinib	0.67	6.3	6.97

Fig 3. Molecular efficacy trials: combinatorial effects on short-term inhibition of the mammalian target of rapamycin and epidermal growth factor receptor signaling pathways. Twelve-week-old RIP1-Tag2 animals were treated with vehicle, rapamycin (5 mg/kg), erlotinib (80 mg/kg), or in combination for 3 days. (A) Apoptosis was analyzed by terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) staining and quantitated (C). Proliferation was similarly analyzed by immunohistochemistry for Ki67 (B) and quantitated (D). The data were from five fields analyzed in two to three tumors each from three mice per treatment group and analyzed by a Mann-Whitney two-tailed test (InStat, GraphPad Software, San Diego, CA). Scale bars, 100 μm. (E) Cell viability was assessed using a laboratory test that measures cell viability, factoring in both cell proliferation and cell death (the MTT assay) for an RIP1-Tag2 tumor-derived pancreatic neuroendocrine tumor cell line, βTC3, treated with vehicle, rapamycin (50 nmol/L dissolved in 95% ethyl alcohol), erlotinib (10 μmol/L dissolved in dimethyl sulfoxide), or the combination of the two for 72 hours. (F) Annexin V/plus propidium iodide flow cytometry was used to assess early (annexin V^+/PI^-) and late (annexin V^+/PI^+) stages of apoptotic cell death of βTC3 cells treated with vehicle, rapamycin (10 nmol/L), erlotinib (10 μmol/L), or the combination for 24 hours.

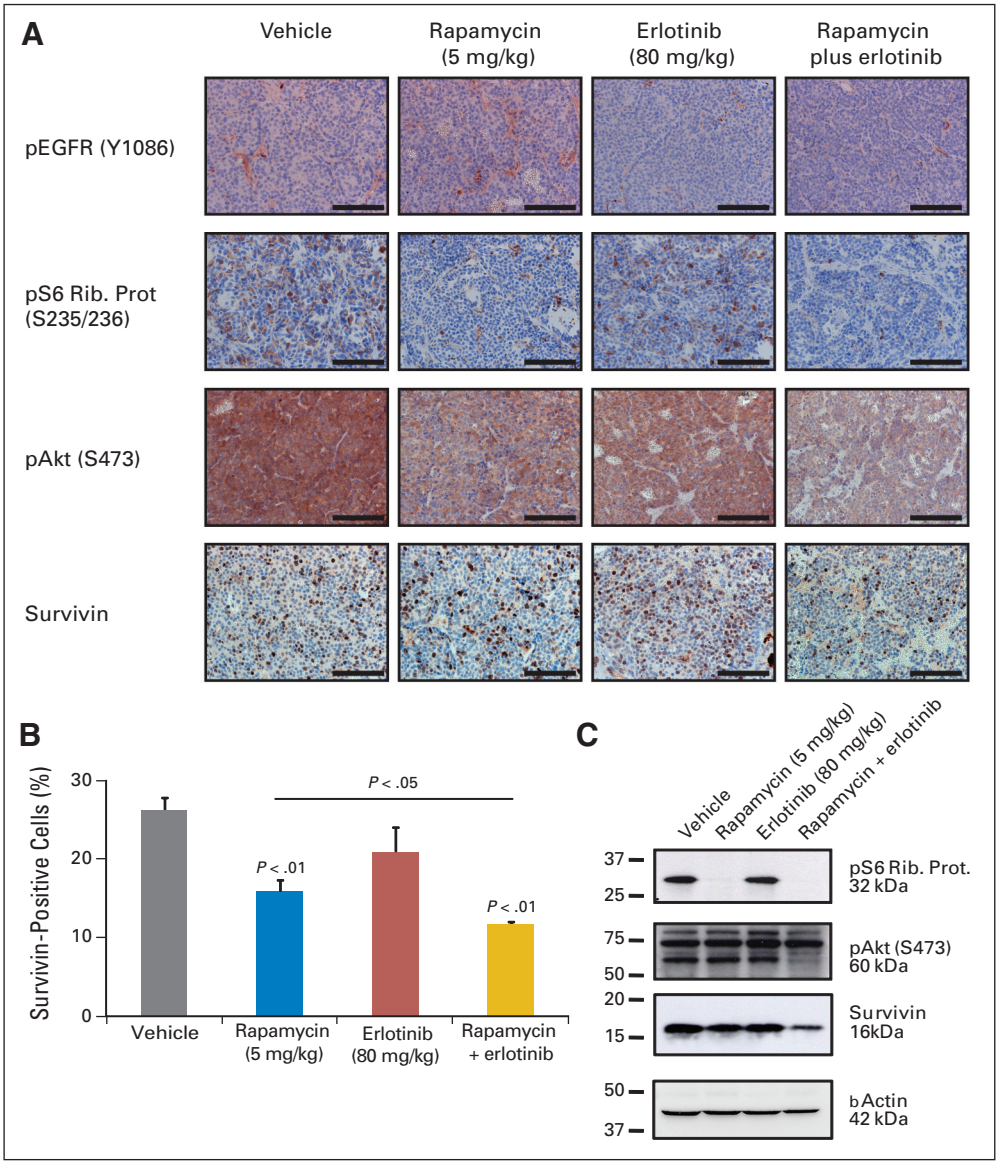


Fig 4. Inhibition of epidermal growth factor receptor (EGFR), mammalian target of rapamycin (mTOR), and Akt and decreased expression of survivin in pancreatic neuroendocrine tumor (PNET) on short-term treatment with erlotinib and rapamycin. Twelve-week-old RIP1-Tag2 animals were treated with vehicle, rapamycin (5 mg/kg), erlotinib (80 mg/kg), or the combination for 3 days. (A) Immunohistochemistry of PNET tissue sections for pEGFR, pS6, and pAkt to assess activation of EGFR, mTOR, and Akt, respectively, and survivin. Scale bars, 100 μm. (B) Survivin was quantitated as the percentage of survivin-positive cells in the tumors. The data were from five fields analyzed in two to three tumors each from three mice per treatment group and analyzed against the vehicle control group by a Mann-Whitney two-tailed test (InStat, GraphPad Software, San Diego, CA). (C) Western blot analysis for pS6, pAkt, and survivin in extracts from PNET of each treatment group.

lead to a significant increase in the frequency of dying cancer cells, whereas erlotinib had no effect (Figs 3A and 3C). The combination produced a significant increase in apoptosis in the tumors, higher than the rapamycin monotherapy. Immunostaining with a marker that labels proliferating cells (Ki67) revealed no effect on proliferation with rapamycin treatment, a trend toward decreased proliferation with erlotinib treatment, and, in notable contrast, the combination produced a significant reduction in proliferation (Figs 3B and 3D).

We next sought to determine whether these drugs were directly impacting the PNET cancer cells, given that both inhibitors have been reported in other contexts to affect the tumor vasculature and inflammatory cells in addition to cancer cells.[6,42-44] To that end, we treated a PNET-derived cancer cell line, βTC3.[45] Consistent with the results in vivo, each inhibitor impaired the viability and increased the frequency of apoptosis in the cultured cancer cells, and the combination was more effective (Figs 3E and 3F).

Immunostaining of tumor tissue revealed effective in vivo inhibition of EGFR and mTOR, both in the cancer cells and in the tumor vasculature (Figs 4A and 4C). However, the Akt kinase, which is downstream from EGFR and upstream of mTOR, was only minimally inhibited by either monotherapy. In marked contrast, dual targeting of the mTOR and EGFR signaling pathways resulted in substantial inhibition of Akt, which was correlated with decreased proliferation (Figs 4A, 3B, and 3D).

Motivated by the observation that the antiapoptotic protein survivin was upregulated in this tumorigenesis pathway, we assessed the effect of the therapies on its expression. Rapamycin produced a significant decrease in survivin expression, which was further decreased by combination therapy (Figs 4A through 4C). Notably, expression of survivin was inversely correlated with the extent of programmed cell death (Figs 3A and 3B), consistent with its ascribed role as a prosurvival factor.

To determine whether survivin could modulate viability of the cancer cells, we used siRNAs against *Birc5* to knockdown gene expression in cultured PNET cancer cells, which produced a significant decrease in viability and increased apoptosis (Data Supplement Fig 1A-1D). Additionally, there was an eight-fold increase in

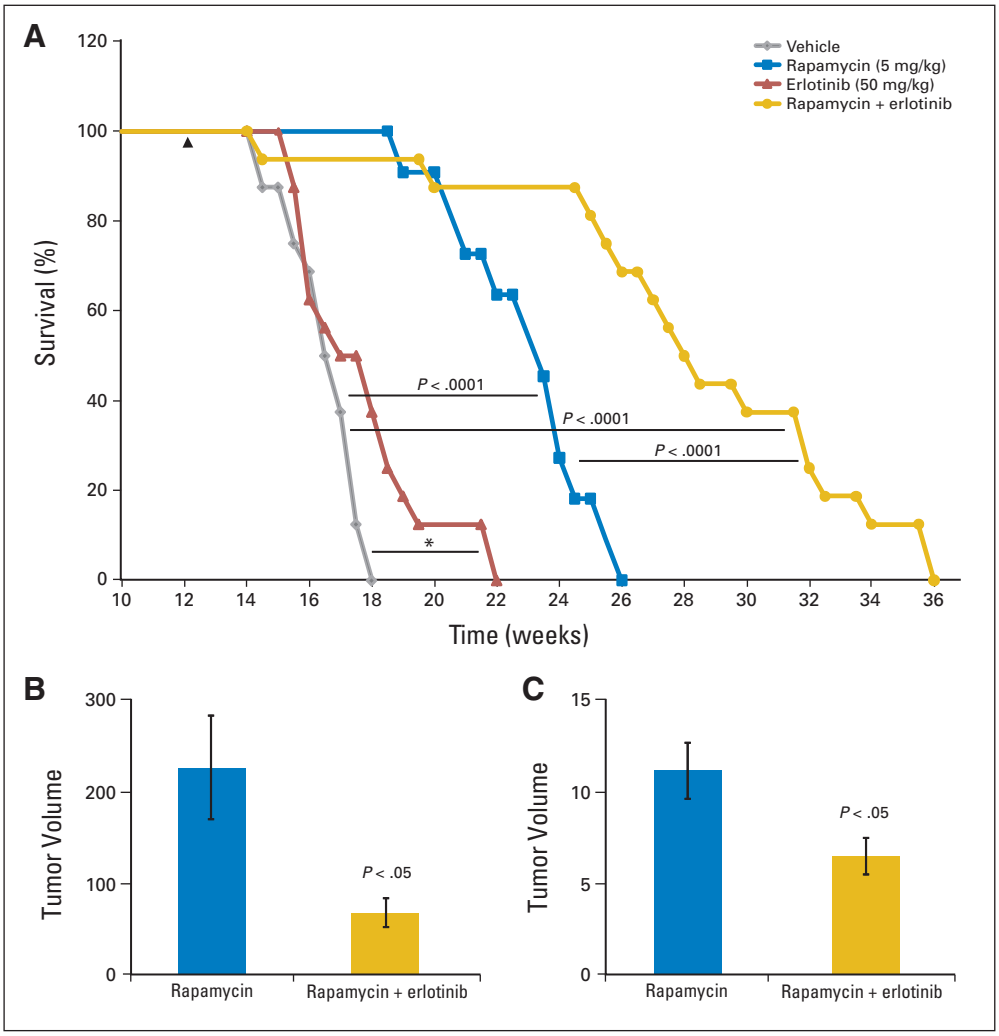


Fig 5. Survival trial with rapamycin and erlotinib in RIP1-Tag2 mice. (A) Survival of mice treated with vehicle (n = 16), rapamycin (5 mg/kg, n = 11), erlotinib (50 mg/kg, n = 16), or rapamycin plus erlotinib (n = 15). The data were analyzed by a log-rank test (from Russell Thompson: The Walter and Eliza Hall Institute of Medical Research, http://bioinf.wehi.edu.au/software/russell/logrank/index.html). Tumor volume (B) and number (C) from mice treated with rapamycin (n = 7; average age, 22.6 weeks) or rapamycin plus erlotinib (n = 8; average age, 26.9 weeks) that were dissected at time of euthanasia. The data were analyzed by a Mann-Whitney two-tailed test (InStat, GraphPad Software, San Diego, CA).

cells at the G_2/M phase of the cell cycle, indicative of cell cycle arrest (Data Supplement Fig 1E).[46,47] Thus survivin has demonstrable functional roles in facilitating progression through the cell division cycle and limiting programmed cell death in PNET cancer cells.

Efficacy Trials Involving Dual Inhibition of the mTOR and EGFR Signaling Pathways

We next performed preclinical trials designed to determine whether this therapeutic combination could produce antitumor responses and prolong the life span of these animals. Initially, we performed a defined end point regression trial[48] on tumor-bearing animals with erlotinib administered at 80 mg/kg. Although treatment with erlotinib produced significant retardation in tumor growth, this dose was toxic when given chronically, resulting in decreased survival and weight loss (Data Supplement Fig 2). When erlotinib was lowered to a well-tolerated dose of 50 mg/kg, tumor growth was still impaired, but with minimal weight loss. Rapamycin was highly efficacious as monotherapy, producing stable disease and no evident toxicity. In combination, the poorly tolerated high-dose erlotinib plus rapamycin led to a significant regression in tumor burden in the treated cohort compared with the starting point and to the monotherapy cohorts. The lower, well-tolerated dose of erlotinib in combination with rapamycin was less effective at reducing tumor burden and survivin expression and at increasing apoptosis (Data Supplement Figs 2 and 3), but the significantly reduced toxicity dictated its use in subsequent trials.

We then performed a survival trial, starting at 12 weeks of age, to assess the benefits of rapamycin, low-dose erlotinib, and the combination. The average survival of vehicle-treated mice was 16.7 weeks of age (Fig 5A). Similar to the results of human trials,[49] erlotinib as monotherapy demonstrated marginal efficacy. In contrast, the rapamycin-treated animals had a significant extension of life (from 16.7 to 22.7 weeks). The combination therapy further extended the average survival from 16.7 to 28 weeks, which was significant even when compared with the already efficacious rapamycin monotherapy.

Tumors were collected whenever possible from moribund animals. The vehicle-treated and erlotinib-only treated animals died shortly after 16 weeks of age and were not analyzed because these tumors were analogous to the defined end point regression trial (Data Supplement Figs 1-3). Therefore, for the survival trial, we only compared tissues between the rapamycin-only and the combination-treated cohorts. The average age at dissection was 22.6 weeks for the

rapamycin-treated group and 26.9 weeks for the combination group. Despite the combination cohort being more than a month older than the rapamycin monotherapy cohort, the tumor burden for the combination-treated animals was three-fold smaller, with lower tumor number (Figs 5B and 5C).

The combination therapy produced significant decreases in proliferation and in survivin expression and an increase in apoptosis as compared with the rapamycin monotherapy group (Appendix Fig A1, online only). It is notable that the decreased survivin expression and elevated cell death were similar to those measured during the regression trial, suggesting that this therapeutic regimen was able to induce long-term stable disease (Appendix Fig A1; Data Supplement Fig 3). Interestingly, the rapamycin monotherapy group showed a significant reactivation of the mTOR pathway, indicative of adaptive resistance to the monotherapy that was obviated by the combination therapy (Data Supplement Fig 4).

DISCUSSION

In this study we used a preclinical mouse model of PNET to evaluate a rational combinatorial therapy regimen targeting the mTOR and EGFR signaling pathways in the treatment of advanced PNET. The logic is based on the hypothesis that EGFR inhibition might counterbalance a form of adaptive resistance involving the loss of feedback inhibition and consequent hyperactivation of the Akt signal-transducing kinase when mTOR signaling is inhibited as a monotherapy.[12-14] Although such resistance was implicated in the eventual relapse to progressive disease, mTOR inhibition was surprisingly effective as monotherapy, reflecting the unexpected responses seen with a rapalog, everolimus, in human clinical trials in PNET, wherein both stable disease and an increase in overall survival was observed.[1] A phase III clinical trial is underway to address the potential use of mTOR inhibition as a first-line therapeutic in PNETs.[1,11] Interestingly, although we envisioned that rapamycin might impair inflammation and/or angiogenesis,[6,8] the primary effect was on the cancer cells, where it elicited increased rates of apoptosis and impaired cell division, with no evident effects on the tumor vasculature (Data Supplement Fig 5).

The modest efficacy seen in the monotherapy trials of the EGFR inhibitor erlotinib is consistent with a recent study investigating EGFR signaling in PNET tumors in RIP-Tag2 mice[20] and additionally reflects the dose reduction to limit toxicity. Notably, EGFR inhibitors, including erlotinib, have presented toxicity issues in clinical trials, presumably reflecting its role in normal organ homeostasis.[49-52] Our data suggest that although EGFR inhibitors may have limited benefit as monotherapy in human PNET, they hold promise when used at lower doses in combination with drugs targeting mTOR, including the rapalogs in late-stage clinical investigation and perhaps also newer generations of inhibitors targeting both of the recently identified mTOR kinase complexes, mTORC1 and mTORC2.[53-55]

In combination, the mTOR and EGFR inhibitors were complimentary, producing remarkable survival benefit and prolonged tumor regression and stasis. The survival trial revealed that although rapamycin monotherapy was surprising efficacious, the tumors eventually became resistant to the monotherapy after a period of stasis/regression, producing large tumors that were highly proliferative with a low rate of apoptosis. In contrast, combinatorial targeting produced long-term suppression of tumor growth and limited development of adaptive resistance in both regression and survival trials.

Searching for biomarkers of response, we found that the combination therapy significantly reduced the otherwise elevated expression of a mitotic progression and antiapoptotic factor, survivin, correlating with increased apoptosis and decreased proliferation in the treated tumors. Inhibition of *Birc5* expression led to a significant decrease in PNET cancer cell viability by increasing apoptosis and inducing cell cycle arrest, consistent with a functional role in PNET. Survivin has been implicated as a biomarker and functional participant by its increased expression in various human cancers; additionally, survivin is known to be upregulated in response to oncogenic signaling, including via mTOR.[31-34,39,56] It will be interesting to determine whether survivin has utility as a biomarker for assessing the efficacy of these and other therapeutics in the treatment of human neuroendocrine cancer.

The results from our preclinical study have motivated the initiation of an ongoing phase II clinical trial designed to evaluate the safety and efficacy of everolimus and erlotinib in patients with low-grade neuroendocrine tumors.[57] In addition to standard safety and efficacy end points, the utility of candidate biomarkers of mTOR and EGFR pathway activity will be investigated. Baseline expression of PTEN, pAkt, p70S6K1, pEGFR, *p*-IRS, *p*-mTOR, and *p*-4E-BP will be assessed in archived tumor samples; the possibility of including survivin expression is currently being explored. The preclinical results predict that combinations of mTOR inhibitors and lower, better-tolerated doses of erlotinib (and other EGFR inhibitors) will have benefit in patients with PNET and perhaps other cancers. Recent preclinical trials suggest analogous benefit in prostate cancer[26] and biliary tract adenocarcinoma.[58] Importantly, not all tumor types are expected to respond to this combination, as clinical trials combining mTOR and EGFR inhibitors have shown equivocal efficacy in glioblastoma[59] and breast cancer.[60] The use of genetically engineered mouse models of other forms of human cancer may allow additional responsive tumor types to be identified, thereby incentivizing clinical trials in such indications, much as illustrated in this prototypical preclinical case study and its initial translation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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AUTHOR CONTRIBUTIONS

Conception and design: Christopher W. Chiu, Douglas Hanahan
Financial support: Hiroaki Nozawa, Douglas Hanahan

Collection and assembly of data: Christopher W. Chiu, Hiroaki Nozawa
Data analysis and interpretation: Christopher W. Chiu, Douglas Hanahan
Manuscript writing: Christopher W. Chiu, Douglas Hanahan
Final approval of manuscript: Christopher W. Chiu, Hiroaki Nozawa, Douglas Hanahan

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Glossary Terms

Apoptosis: Also called programmed cell death, it is a signaling pathway that leads to cellular suicide in an organized manner. Several factors and receptors are specific to the apoptotic pathway. The net result is that cells shrink, develop blebs on their surface, and their DNA undergoes fragmentation.

Biomarker: A functional biochemical or molecular indicator of a biologic or disease process that has predictive, diagnostic, and/or prognostic utility.

EGFR (epidermal growth factor receptor): Also known as HER1, EGFR belongs to a family of receptors (HER2, HER3, HER4 are other members of the family) and binds to the EGF, TGF-α, and other related proteins, leading to the generation of proliferative and survival signals within the cell. It also belongs to the larger family of tyrosine kinase receptors and is generally overexpressed in several solid tumors of epithelial origin.

Erlotinib: Also known as Tarceva, erlotinib is a small molecule that inhibits the tyrosine kinase activity of EGFR/HER1 and has been evaluated extensively in clinical trials in patients with non–small-cell lung cancer, pancreatic cancer, and glioblastoma multiforme.

IAP (inhibitors of apoptosis proteins): IAPs suppress host cell death in response to viral infection. By binding to caspases, they directly inhibit apoptosis. Survivin and xIAP are members of this family, differing perhaps in binding to selective caspases.

mTOR: The mammalian target of rapamycin belongs to a protein complex (along with raptor and GβL) that is used by cells to sense nutrients in the environment. mTOR is a serine/threonine kinase that is activated by Akt and regulates protein synthesis on the basis of nutrient availability. It was discovered when rapamycin, a drug used in transplantation, was shown to block cell growth presumably by blocking the action of mTOR.

Akt/PKB: Protein kinase B belongs to a pathway that is responsible for cell survival. Following activation (ie, phosphorylation) by PI3K, activated Akt blocks the activity of molecules involved in the apoptotic pathway by in turn phosphorylating them.

Rapamycin: A potent immunosuppressant used in transplantation that inhibits cell cycle progression, preventing cell proliferation.

Survivin: IAPs suppress host cell death in response to viral infection. By binding to caspases, they directly inhibit apoptosis. Survivin and xIAP are members of this family, differing perhaps in binding to selective caspases.

Pancreatic neuroendocrine tumor (PNET/Islet Cell Tumor): A relatively rare form of pancreatic cancer, in which tumors arise from the pancreatic islets of Langerhans, being composed of transformed islet cells that produce (or once produced) insulin or other polypeptide hormones. The endocrine hormone–secreting islet cells (and islet tumors) express a number of neuronal genes, reflecting an evolutionary heritage with neuronal cells and hence their designation as neuroendocrine, to contrast them to the prevalent form of human pancreatic cancer, ductal adenocarcinoma.